Preformulation Analysis and Product Stability of Norelgestromin/Ethinyl Estradiol Intravenous Infusion

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Introduction

Contraception is achieved mainly by inhibiting ovulation through the combined activity of two main components: estrogen and progestin. Norelgestromin/ethinyl estradiol (NE/EE) is a progestin/estrogen combination hormonal contraceptive indicated for the prevention of pregnancy in women. The intravenous (IV) route allows 100% bioavailability and is the most rapid method of getting a drug into systemic circulation. Development of an IV formulation that contains both NE and EE is important to determine the absolute bioavailability of both NE and EE after administration of other pharmaceutical products (e.g., contraceptive transdermal delivery systems). The very poor solubility and wettability of these drugs, along with their high potency (adsorption issues), gave rise to difficulties in designing an IV pharmaceutical formulation that could be used in clinical bioavailability studies. In this study, we developed and validated HPLC chromatographic methods for quantification of both drugs, we used a cosolvent/surfactant system (ethanol/polysorbate 80) to solubilize NE and EE. We optimized the composition of the solvent system to minimize the amount of both ethanol and polysorbate 80 while maintaining therapeutically effective amounts of both drugs. In addition, we evaluated the stability of NE/EE in an IV delivery bag to ensure a practical shelf life suitable for use in a clinical bioavailability study.

Methods

Solubility studies

Solubility studies were conducted to optimize the formulation of NE/EE IV solution. Solubility measurements were determined in various solvents:

- Normal saline
- Normal saline + 10% ethanol
- Sterile water for injection
- Sterile water for injection with 1 - 10 % ethanol
- Sterile water for injection with 2.5 % ethanol and 2.5 % polysorbate 80

Amounts of NE and EE were weighed in glass vials containing 60 mL of solvent. The samples were shaken at 25 ± 2°C for 24 h, and then filtered through 0.2 µm filter. Concentrations of dissolved NE and EE were analyzed using the developed and validated HPLC method.

Chromatographic conditions

- ***Method A:*** High-performance liquid chromatography (HPLC) column used was the Symmetry® C_{18} (4.6 mm x 150 mm, 5 µm) (Waters®; Milford, MA) coupled with Phenomenex Luna® Security C_{18} guard column (4.0 mm x 3.0 mm). Mobile phase composition was (A): methanol, (B): acetonitrile and (C):water. Isocratic elution (A:50, B:20, C:30, v/v) was employed at a flow rate of 0.5 mL/min. Column and autosampler temperatures were set at 60°C and 25°C, respectively. Injection volume of 20 µL was used.

- ***Method B:*** Similar to Method A but mobile phase composition was; (A):acetonitrile and (B):water containing 0.1 % v/v triethylamine adjusted to pH=6.6 (A:35, B:65, v/v) at flow rate of 1.5 mL/min. Injection volume of 50 µL was used.

- Robustness testing for both NE and EE was done by the use of design of experiment (DOE) approach using Plackett–Burman design. Chromatograms and 3 D plots were generated by Empower™ software (Waters®; Milford, MA). The experimental results were computed using MODDE pro 11 (Umetrics, Sweden) with respect to capacity factor (*K*) and number of theoretical plates (N).

Intravenous solution preparation and stability studies

NE/EE IV solution was prepared with sterile water for injection with 2.5% ethanol and 2.5% polysorbate 80 as a cosolvent/surfactant system to obtain a final drug solution of 252 µg of NE and 25 µg EE and from a concentrated stock drug solution (5X). Concentrated stock solutions and IV solutions (Baxter Intravia® medication delivery bag) were stored in the refrigerator (3.7°C ± 0.6) and at room temperature (19.5°C ± 0.5), respectively. Additional studies were conducted to examine stability of the IV solution using the IV administration set (Alarias® low sorbing set) with and without an inline filter. The solution was allowed to drip at 1 mL/min over a 60 min period. Samples were obtained at the beginning, middle and end of the 60 min duration. Chemical stability was evaluated for up to 10 days. NE and EE concentration, purity, and degradant levels were determined using a stability indicating HPLC method.

Results

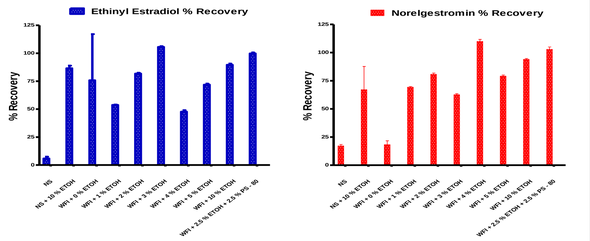

Figure 1. Solubility of NE and EE in different solvent systems . Data represent mean ± SD (n=3) (NS: Normal saline, WFI: Sterile water for injection, ETOH: Ethanol, PS-80: Polysorbate 80)

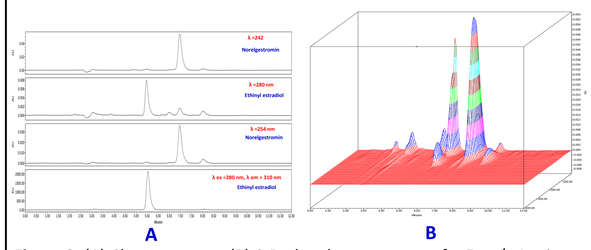

Figure 2. (A) Chromatogram, (B) 3 D plot chromatogram of a 5 µg/mL mixture of NE and EE indicating polysorbate 80 interference ***(Method A)***

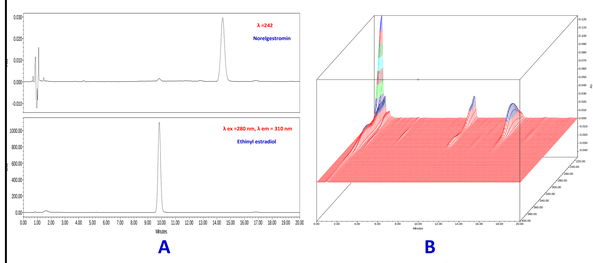

Figure 3. (A) Chromatogram , (B) 3 D plot chromatogram of a 5 µg/mL mixture of NE and EE indicating absence of polysorbate 80 interference ***(Method B)***

Table 1. Summary of validation and regression equation parameters of the reversed phase liquid chromatographic method for determination of NE and EE

	Ethinyl estradiol	Norelgestromin
Linearity:		
Regression equation	Y = 28330000 x – 16186.3	Y = 115413 x – 819.8
Correlation Coefficient (r)	0.9998	0.9995
Range	0.1 – 50 µg/mL	0.1 – 50 µg/mL
Limit of Detection (LOD)	0.015 µg/mL	0.007 µg/mL
Limit of Quantitation (LOQ)	0.046 µg/mL	0.022 µg/mL
Precision:		
Repeatability (Intraday) (% RSD)		
LLOQ (0.1 µg/mL)	4.49 %	1.97 %
QCL (0.3 µg/mL)	2.05 %	2.53 %
QCM (3 µg/mL)	1.42 %	0.74 %
QCH (30 µg/mL)	1.99 %	3.11 %
Intermediate precision (Interday) (% RSD)		
LLOQ (0.1 µg/mL)	4.76 %	0.66 %
QCL (0.3 µg/mL)	3.88 %	0.77 %
QCM (3 µg/mL)	1.04 %	0.73 %
QCH (30 µg/mL)	0.72 %	2.29 %
Accuracy:		
(Mean ± SD)		
LLOQ (0.1 µg/mL)	102.91 ± 4.49	100.53 ± 1.98
QCL (0.3 µg/mL)	102.63 ± 2.10	101.59 ± 2.57
QCM (3 µg/mL)	101.48 ± 1.44	101.69 ± 0.75
QCH (30 µg/mL)	98.29 ± 1.96	101.67 ± 3.16

LLOQ: Lower limit of quantitation, QCL: Quality control low, QCM: Quality control medium, QCH: Quality control high, RSD: Relative standard deviation, SD: Standard deviation

Table 2. Design of experiment for robustness testing of NE and EE

					NE	EE	
Exp. No	**Column temperature**	**Flow rate**	**Acetonitrile**	**Water**	**Wavelength**	**Excitation wavelength**	**Emission wavelength**
1	58	1.3	30	70	243	281	309
2	62	1.7	30	70	241	279	311
3	58	1.3	40	60	241	279	309
4	62	1.7	40	60	243	281	309
5	58	1.3	30	70	243	281	311
6	62	1.7	30	70	241	279	311
7	58	1.3	40	60	241	279	311
8	62	1.7	40	60	243	281	309
9	**60**	**1.5**	**35**	**65**	**242**	**280**	**310**
10	**60**	**1.5**	**35**	**65**	**242**	**280**	**310**
11	**60**	**1.5**	**35**	**65**	**242**	**280**	**310**

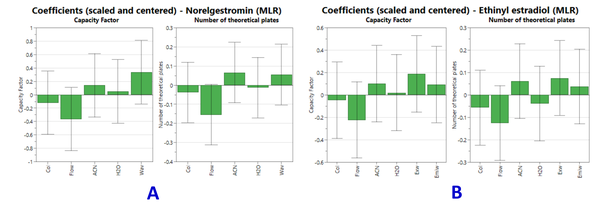

Figure 4. Regression coefficient plots obtained following Plackett-Burman design using MODDE pro 11 (Umetrics, Sweden) : (A) NE and (B) EE

Table 3. Stability of EE during storage in glass bottles (concentrated stock solution) and in sterile medication delivery bags (Intravia®, Baxter) (IV solution)

Time (h)	Concentrated Stock Solution#	IV solution#
0	100.90 ± 1.64	100.84 ± 2.45
0.5	100.04 ± 1.94	100.49 ± 1.59
1	100.21 ± 1.27	100.45 ± 1.85
2	99.96 ± 1.89	99.69 ± 2.05
4	99.82 ± 1.76	98.62 ± 2.17
6	99.64 ± 2.46	98.65 ± 1.46
8	98.76 ± 1.09	98.81 ± 1.73
10	98.70 ± 2.01	97.64 ± 2.25
12	98.30 ± 2.86	95.07 ± 0.50
24	96.16 ± 2.51	94.97 ± 3.86
48	94.12 ± 2.73	94.17 ± 4.79
72	93.49 ± 1.51	94.45 ± 2.65
96	92.28 ± 1.31	94.32 ± 2.79
120	92.41 ± 1.61	93.35 ± 1.15
144	91.44 ± 2.21	93.24 ± 2.64
168	91.98 ± 1.82	93.14 ± 2.72
192	91.68 ± 1.43	91.33 ± 4.74
216	91.81 ± 2.96	92.72 ± 2.61
240	84.24 ± 1.43	86.84 ± 2.18

#Expressed as percentage of original concentration remaining (Mean ± SD, n = 3)

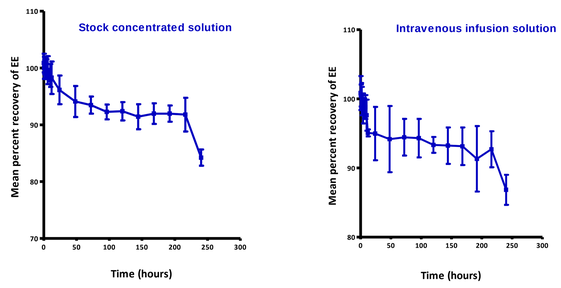

Figure 5. Plot of mean percent recovery of EE (mean ± SD, n = 3) versus time for concentrated stock and IV solution

Table 4. Stability of NE during storage in glass bottles (concentrated stock solution) and in sterile medication delivery bags (Intravia®, Baxter) (IV solution)

Time (h)	Concentrated Stock Solution#	IV solution#
0	98.49 ± 1.23	98.79 ± 1.92
0.5	98.54 ± 1.99	98.25 ± 1.88
1	98.22 ± 1.79	98.21 ± 2.56
2	98.14 ± 0.49	98.29 ± 2.11
4	98.29 ± 0.53	98.76 ± 3.69
6	98.98 ± 2.75	98.58 ± 3.24
8	98.88 ± 0.79	98.85 ± 2.65
10	98.59 ± 1.44	97.14 ± 2.29
12	98.92 ± 2.30	97.68 ± 1.55
24	98.25 ± 1.74	97.68 ± 1.55
48	98.07 ± 2.28	97.31 ± 5.41
72	97.84 ± 1.27	96.68 ± 2.34
96	94.87 ± 1.09	95.84 ± 3.29
120	92.25 ± 1.01	95.79 ± 1.20
144	92.25 ± 3.00	93.83 ± 4.76
168	92.13 ± 2.98	93.79 ± 2.50
192	91.63 ± 2.45	91.60 ± 3.72
216	89.95 ± 2.39	90.99 ± 2.51
240	83.41 ± 4.63	90.01 ± 2.89

#Expressed as percentage of original concentration remaining (Mean ± SD, n = 3)

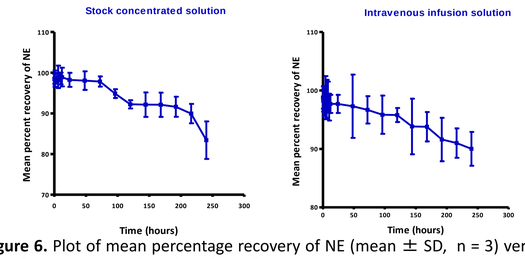

Figure 6. Plot of mean percentage recovery of NE (mean ± SD, n = 3) versus time for concentrated stock and IV solution

Table 5. Physical comptability testing of the intravenous administration set

	No inline filter		Inline filter	
	EE	NE	EE	NE
	(Mean ± SD, n=3)	(Mean ± SD, n=3)	(Mean ± SD, n=3)	(Mean ± SD, n=3)
Beginning	102.74 ± 4.70	100.33 ± 6.71	96.99 ± 2.94	99.07 ± 0.002
Middle	100.03 ± 4.15	100.94 ± 4.04	95.94 ± 0.83	99.82 ± 0.004
End	96.34 ± 3.26	99.20 ± 5.28	97.39 ± 1.69	98.09 ± 4.02

Conclusions

- An IV formulation of NE and EE was developed using 2.5% ethanol and 2.5% polysorbate 80 as a cosolvent/surfactant system.
- Our stability studies indicated that at room temperature the IV formulation was chemically stable up to 9 days when stored in Intravia® medication delivery bags.
- In addition, our stability studies indicated that both drugs were compatible with Alarias® low sorbing IV administration set (i.e., stable with minimal drugs adsorption).
- A new chromatographic method was developed and validated for the analysis of both compounds in presence of polysorbate 80 which usually causes interference during chromatographic analysis.
- This IV formulation can be used for future clinical bioavailability studies to determine the absolute bioavailability of other pharmaceutical formulations containing NE and EE (e.g., contraceptive transdermal systems).

Acknowledgment

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